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CERTIFICATE OF MAILING

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ELI LILLY AND COMPANY

By James W. Burton Date 6-17-02

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	: Tracy Leitner Borts, *et al.*	)
Serial No.	: 09/091,605	)
Filed	: June 16, 1998	) Group Art Unit: 1600
Title	: Diabetes Therapy	) Examiner: G. Lee
Docket No.	: X-9872	)

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JUN 17 2002
OFFICE OF PETITIONS

June 10, 2002

EXHIBIT A

DECLARATION OF ANNE REIFEL MILLER UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Arlington, VA 22202

Sir:

I, Anne Reifel Miller, of the City of Indianapolis, County of Marion, State of Indiana, on information and belief, state and declare that:

1. I am a co-inventor of the invention described and claimed in the above-identified patent application Serial No. 09/091,605 (hereinafter the '605 Application). I have reviewed the '605 Application and the Final Office Action for this

Conder RDS 3/25/03

application. In view of this Office Action, I submit herewith an exact report on experiments I directed in connection with the above-identified application.

2. The '605 Application shows that 293 cells were used to create a GLP-1-expressing stable cell line and that these transformed cells were immunologically masked and introduced into diabetic rats for the purpose of expressing GLP-1 in the rats.

3. Under my direction, data was obtained from diabetic rats that were transplanted with 2×10^7 cells of an immunologically masked, stable mammalian cell line expressing a GLP-1 analog (Val^8-GLP-1) and compared to control diabetic rats transplanted with 293 cells not expressing the GLP-1 analog and mock-transplanted control diabetic rats into which no cells were transplanted.

4. Data demonstrating increased insulin levels and decreased blood glucose levels in mammals transplanted with immunologically masked cells from a stable cell line expressing Val^8-GLP-1 were obtained as follows:

· 293 cells were transfected with a vector capable of expressing Val^8-GLP-1 and possessing a HygB resistance gene.

· Stable cell lines expressing GLP-1 were expanded from HygB resistant clones.

· The secretion levels of active Val^8-GLP-1 by individual clones were evaluated by testing their

culture supernatants in a biological assay that measures luciferase expression in 293 cells stably co-transfected with GLP-1 receptor and a luciferase reporter gene under the control of a cyclic AMP response element. The Val^8-GLP-1 secretion levels were determined to be elevated as shown in Table 1 below.

· F(ab')2 fragments were generated from anti-HLA class I antibodies by digestion with pepsin and purified using an immobilized protein A column followed by gel filtration.

· To prevent immune rejection, 2 x 10^7 stable, transformed cells expressing Val^8-GLP-1 were immunologically masked by incubating with the purified F(ab')2 fragments prior to transplantation for the purpose of preventing the host cytotoxic T-cells from interacting with the transplanted cells.

· The immunologically masked cells were transplanted under the renal capsule of 8-week old male Zucker Diabetic Fatty (ZDF) rats, a well validated animal model of diabetes.

· Blood samples were taken from their tails at intervals of 1-2 times per week for measurement of plasma glucose and plasma insulin levels which demonstrated increased insulin levels and decreased blood glucose levels in the rats transplanted with cells of the Val^8-GLP-1 expressing stable cell line (see Appendix A and B attached).

Table 1 Levels of Val^8-GLP-1 Secretion in Culture

Clone #	Protein, μg	Light Units	L.U./Protein
2	247.45	95.23	0.39
11*	147.71	187.88	1.27
12	189.60	132.58	0.70
22	223.51	149.18	0.67
23	150.70	232.98	1.55
24	189.60	21.17	0.11
27	239.47	164.44	0.69
293 control	303.31	2.88	0.01

* expanded and used in transplants

5. Cells stably transfected with a vector capable of expressing Val^8-GLP-1 resulted in an increase of secreted Val^8-GLP-1 (Table 1). When these cells were immunologically masked and transplanted into diabetic rats, there resulted a statistically significant decrease in plasma glucose levels compared to those rats that were mock transplanted (Hanks) or transplanted with control cells (293 cells) that do not express Val^8-GLP-1, demonstrating that the transplanted cells expressed Val^8-GLP-1 in the rats (Appendix A).

Furthermore, the rats transplanted with these cells had a statistically significant increase in plasma insulin levels compared to those rats mock transplanted or transplanted with control cells that do not express Val^8-GLP-1, again demonstrating that the transplanted cells expressed Val^8-GLP-1 in the rats (Appendix B).

This data is consistent with and corroborates the assertions in the '605 Application that when mammalian cell lines are stably transformed with a vector expressing GLP-1 (or an analog thereof), they

secrete GLP-1 (or analog thereof); and when such cell lines are immunologically masked and transplanted into mammals, they express GLP-1 (or analog thereof), resulting in an increase in plasma insulin levels and a decrease in plasma glucose levels as would benefit a patient with Type II diabetes.

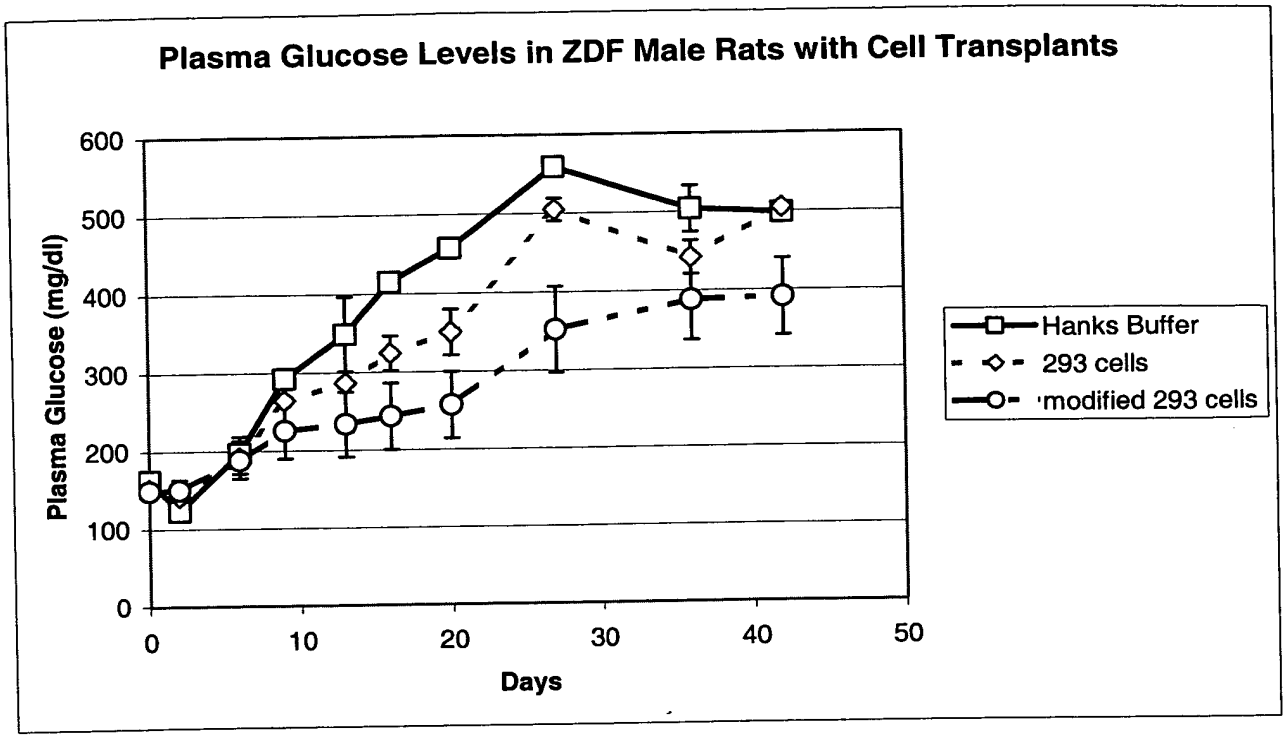
7. I further declare that all statements made in this Declaration are of my own knowledge and are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

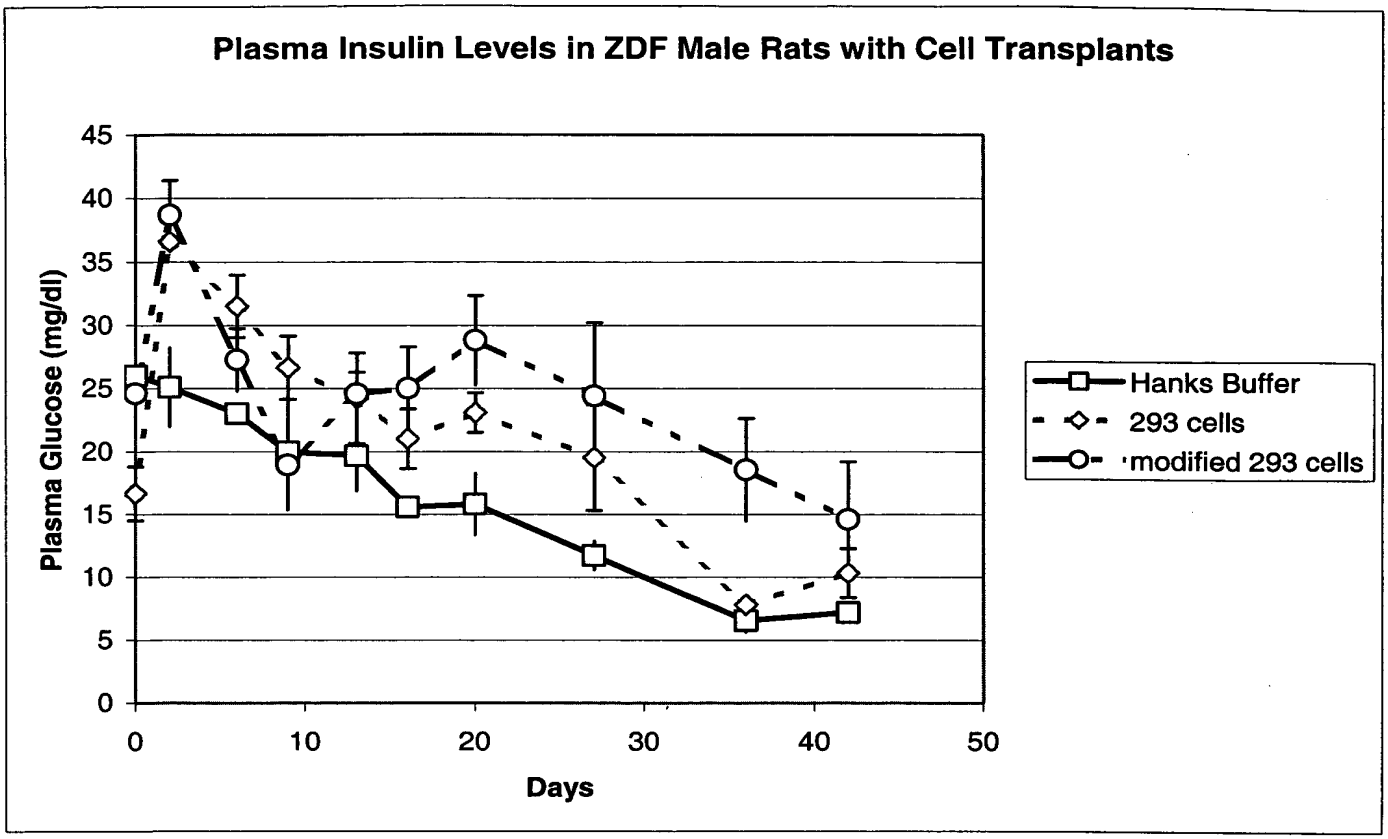
Date: 13 June 02

Anne Reifel Miller

APPENDIX A


Plasma Glucose Levels in ZDF Male Rats with Cell Transplants
Plasma Glucose (mg/dl)
600
500
400
300
200
100
0
0
10
20
30
40
50
Days
Hanks Buffer
293 cells
modified 293 cells

APPENDIX B


Plasma Insulin Levels in ZDF Male Rats with Cell Transplants
Plasma Glucose (mg/dl)
45
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
Days
Hanks Buffer
293 cells
modified 293 cells